Diagnocure Leptospira IgG/IgM Combo Rapid Test - Cassette (Serum / Plasma)

DIAGNOCURE (INDIA)™ In vitro Diagnostic

INTENDED USE

The *Diagnocure* Leptospira IgG/IgM Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM antibodies to *Leptospira interrogans* (*L. interrogans*) in human serum, plasma or whole blood. It is intended to be used as a screening test and as an aid in the diagnosis of infection with *L. interrogans*. Any reactive specimen with the *Diagnocure* Leptospira IgG/IgM Combo Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with hot and humid climates. The natural reservoirs for leptospirosis are rodents as well as a large variety of domesticated mammals. Human infection is caused by *L. interrogans*, the pathogenic member of the genus of *Leptospira*[1,2]. The infection is spread via urine from the host animal.

After infection, leptospires are present in the blood until they are cleared approximately 4 to 7 days after the onset of the disease following the production of anti-*L. interrogans* antibodies, initially of the IgM class. Culture of the blood, urine and cerebrospinal fluid is an effective means of confirming the diagnosis during the 1st and 2nd weeks after exposure. Serological detection of anti-*L. interrogans* antibodies is also a common diagnostic method. Tests available under this category include: 1) The microscopic agglutination test (MAT)[3]; 2) ELISA[4-5]; 3) Indirect fluorescent antibody tests (IFATs)[6]. However, all above mentioned methods require a sophisticated facility and well-trained technicians.

The *Diagnocure* Leptospira IgG/IgM Combo Rapid Test is a simple serological test that utilizes antigens from *L. interrogans* and detects IgG and IgM antibodies to these microorganisms simultaneously. The test can be performed by untrained or minimally skilled personnel without cumbersome laboratory equipment, and the result is available within 15 minutes.

TEST PRINCIPLE

The *Diagnocure* Leptospira IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *L. interrogans* antigens conjugated with colloidal gold (Leptospira conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test lines (M and G lines) and a control line (C line). The M line is pre-coated with monoclonal anti-human IgM for the detection of anti-*L. interrogans* IgM, G line is pre-coated with reagents for the detection of anti-*L. interrogans* IgG, and the C line is pre-coated with goat anti rabbit IgG.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. IgM anti-*L. interrogans,* if present in the specimen, will bind to the Leptospira conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM antibody forming a burgundy colored M line, indicating a *L. interrogans* IgM positive test result.

IgG anti-*L. interrogans,* if present in the specimen, will bind to the Leptospira conjugates. The immunocomplex is then captured on the membrane by the pre-coated reagents forming a burgundy colored G line, indicating a *L. interrogans* IgG positive test result.

Absence of any test lines (M and G) suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugates regardless of color development on any of the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. Sample diluent (3 bottle, 2.5 mL)
4. One package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C - 30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimens for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2°C - 30°C. If stored at 2°C - 8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to over 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by veinpuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into a new pre-labeled tube.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 4: Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C - 8°C if not tested immediately for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipermia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

ASSAY PROCEDURE

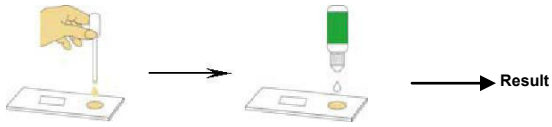
Step 1: Bring the specimen and the test components to room temperature if refrigerated or frozen. Mix the specimen well, prior to assay, once thawed.

Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with the specimen's ID number.

Step 4: Fill the plastic dropper with the specimen.

Immediately add 1 drop (about 30-35 µL) of Sample Diluent to the sample well
Then add 1 drops (about 30-35 µL) of Sample Diluent immediately



1 drop of specimen **1 drop of sample diluents wait for 15 minutss**

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as soon as 1 minute.

Do not read the result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

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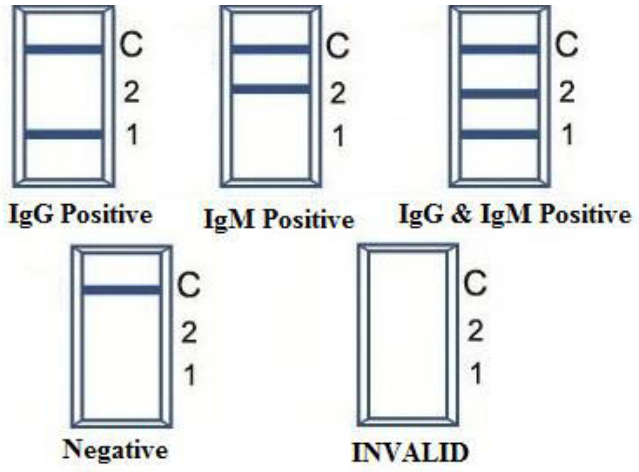
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the whole procedure and repeat the test with a new device.
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits are used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kits fall outside of 2°C - 30 C.
   e. The temperature of the test area falls outside of 15°C - 30 C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT



PERFORMANCE CHARACTERISTICS

1. Clinical Performance For IgM Test

A total of 210 samples from susceptible subjects were tested by the *Diagnocure* Leptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira IgM EIA kit. Comparison of the results for all subjects is shown in the following table.

	***Diagnocure* Leptospira IgG/IgM Combo Rapid Test**		
IgM EIA	**Positive**	**Negative**	**Total**
Positive	9	1	10
Negative	2	198	200
Total	11	199	210

Relative Sensitivity: 90.0%, Relative Specificity: 99.0%, Overall Agreement: 98.6%

2. Clinical Performance For IgG Test

A total of 206 samples from susceptible subjects were tested by the *Diagnocure* Leptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira IgG EIA kit. Comparison of the results for all subjects is shown in the following table.

	***Diagnocure* Leptospira IgG/IgM Combo Rapid Test**		
IgG EIA	**Positive**	**Negative**	**Total**
Positive	6	0	6
Negative	2	198	200

Relative Sensitivity: 100%, Relative Specificity: 99.0%, Overall Agreement: 99.0%

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation Assay Result sections must be followed closely when testing for the presence of antibodies to pathogenic *L. interrogans* in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Diagnocure* Leptospira IgG/IgM Combo Rapid Test is limited to the qualitative detection of antibodies to *L. interrogans* in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable *L. interrogans* antibodies. However, a negative test result does not preclude the possibility of exposure to *L. interrogans*.
4. A negative result can occur if the quantity of *L. interrogans* antibodies present in the specimen is below the detection limits of the assay or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.